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Hypothesis

Pseudouridine in the large-subunit (23 S-like) ribosomal RNA

The site of peptidyl transfer in the ribosome?

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On evolutionary grounds, it has been advocated for more than 40 years that RNA generally, and more recently rRNA in particular, may participate, catalytically, in protein biosynthesis. A specific molecular mechanism has never been proposed. We suggest here that the N-1 position(s) in one or more of the ~4 pseudouridine (ψ) residues in *E. coli* 23 S rRNA catalyzes transfer of the aminoacyl moiety from the 3'-terminus of peptidyl tRNA in the P site to aminoacyl tRNA in the A site of the ribosome. Evidence that supports the proposal in the case of *E. coli* ribosomes, and relevant information pertaining to eukaryotic ribosomes, is summarized. Essential features of the evidence are that (i) the N-1 position in 1-acetylthymine (a direct analogue of 1-acetylpseudouridine) has an especially high potential for acyl-group transfer, comparable to that found for *N*-acetylimidazole (Spector, L.B. and Keller, E.B. (1958) J. Biol. Chem. 232, 185–192), (ii) most of the ψ residues in prokaryotic 23 S rRNA are confined to the peptidyl transferase center in *E. coli* ribosomes, and (iii) Um-Gm-ψ, the most densely modified sequence in eukaryotic 26 S rRNA, is universally conserved at a fixed site in the putative peptidyl transferase center of all eukaryotic ribosomes.

Protein biosynthesis; Peptidyl transfer; Ribosome; rRNA; Modified nucleotide; Pseudouridine

1. INTRODUCTION

Early ideas about the catalytic participation of RNA in its own biodegradation [1–5] had been largely forgotten when the discovery of 'ribozymes' (see [6]) clearly showed that RNA can directly mediate phosphodiester bond cleavage and formation. Likewise, nearly four decades have passed since long-forgotten evidence was first adduced in support of the idea that RNA might participate, catalytically, in the biosynthesis of proteins. Although the early evidence [7] of direct RNA participation in peptide-bond formation [8] was found wanting [9], the reasons that spawned the notion have continued to be germane and attractive, and they have been forcefully articulated and advocated [10–16], garnering particular support more recently from the demonstration that RNA can act as an enzyme [17]. In this report, we adduce historical and experimental evidence to support the idea that pseudouridine residues found in Domains II, IV and/or V of large-subunit (LSU; 23 S-like) rRNA could be peptidyl transfer(ase) sites in the ribosome.

Abbreviations: LSU, large subunit of the ribosome; LSU rRNA, the large (23 S–26 S) rRNA of the large ribosomal subunit; ψ, pseudouridine (5-ribosyluracil)

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2. DISCUSSION

In the same year that a "fifth mononucleotide" [18] was reported to be present in RNA, Spector and Keller [19] submitted a paper on the transacetylation properties of 1-acetyluracil (Fig. 1A). Spector and Keller were studying acetylation of the classical bases in RNA as models of aminoacylation. The notable transacetylating property of 1-acetyluracil was not initially thought of in relation to RNA because it was not then known that such a potential acylation site existed in RNA, i.e. N-1 in uracil is ordinarily a site of *N*-glycosylation in RNA (Fig. 1B). It was soon proposed [20,21] and then shown definitively [22,23], that the fifth mononucleoside in RNA, now known as pseudouridine (ψ) [22], is 5-ribosyluracil (Fig. 1C). Because pseudouridine has its glycosyl attachment at C-5, the N-1 position in the base of ψ is available as a group-transfer site and can be viewed as a potential site for acyl transfer, as in thymine, a direct analogue (of ψ) whose 1-acetyl derivative (Fig. 1D) shares the notable acyltransfer property of 1-acetyluracil [19].

Because it is present in greater quantity in tRNA than in rRNA, we first considered that ψ in tRNA, often

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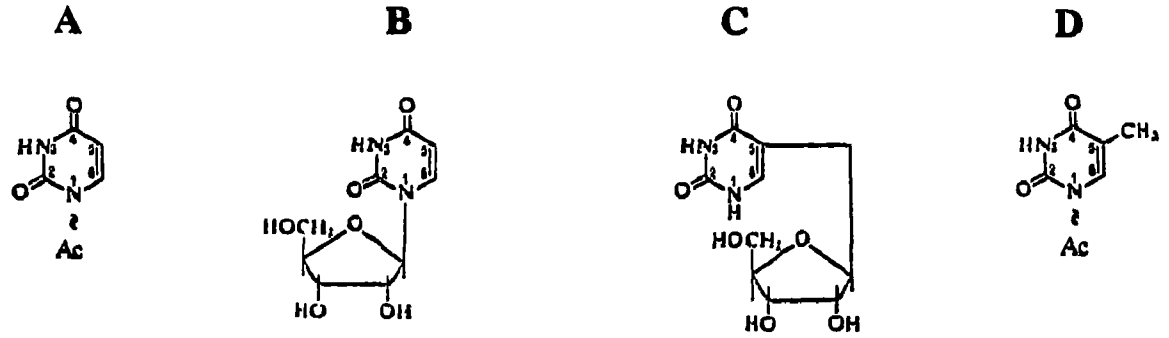

Fig. 1. Structural formulae of 1-acyluracil (A), uridine (B), pseudouridine (C) and 1-acylthymine (D) [19]. The bond between N-1 and the acyl group (Ac) is shown as ~ to emphasize the high group-transfer potential [62] of an acyl substituent at position N-1 [19].

found in a common pentanucleotide sequence (G-m^{5}U-ψ-C-G) [24], might be involved with peptidyl transfer. However, with the discovery of the hypermodified sequence, Um-Gm-ψ, in wheat embryo rRNA [25], our attention was re-directed toward the complement of ψ in wheat rRNA [26]. 'Universal' localization of the Um-Gm-ψ sequence to the LSU rRNA of eukaryotes [27–32], including wheat [33], was especially interesting, because the large subunit is the site of peptidyl transferase activity in the ribosome [34,35]. In allied work with bacterial (*E. coli*) rRNA, we showed that there are three $O^{2'}$-methylated 'dinucleotide sequences' (Gm-G, Cm-C and Um-G) but no $O^{2'}$-methylated 'trinucleotide sequences' in LSU rRNA [36].

Baer and Dubin [37] posited that the Um-G sequence in *E. coli* LSU rRNA, and a similar sequence (Um-Gm-U) in hamster mitochondrial LSU rRNA, are homologous with the Um-Gm-ψ sequence in eukaryotic (nucleocytoplasmic) LSU rRNAs, and that all occur in a universally conserved hairpin loop in the peptidyl transferase domains of LSU rRNAs. From the standpoint that N-1 in ψ might be proximal to ribosomal peptide-bond formation, it was intriguing to find that Um-Gm-ψ^{4460}-U (*Homo sapiens*) [38,39] in eukaryotic LSU rRNAs is homologous with Um-G-U-ψ^{2555} [40] in a corresponding hairpin loop (Fig. 2) that is known to be proximal to the peptidyl transferase centre in Domain V of *E. coli* LSU rRNA [41].

This suggested that N-1 in ψ^{2555} (*E. coli*) or ψ^{4460} (*Homo sapiens*) might mediate peptidyl transfer between tRNAs in the P and A sites of prokaryotic and eukaryotic (nucleocytoplasmic) ribosomes, respectively. Because evidence for a ψ residue at position 2555 in *E. coli* LSU rRNA is problematic (H.F. Noller and C.R. Woese, personal communications), it is important to note that other ψ residues, at position 746 in Domain II [42,43], and at positions 1911 and 1917 in Domain IV [44–46], have also been localized to the peptidyl transferase region of the *E. coli* LSU rRNA [40,47–50]. There is evidence for a single ψ residue in yeast [51] and hamster [52] mitochondrial LSU rRNAs, although the site of ψ in these mitochondrial rRNAs is not known.

The parent (unmodified) U-G-U-U in *E. coli* LSU rRNA, which corresponds to Um-Gm-ψ-U in all eukaryotic nucleocytoplasmic LSU rRNAs, is not conserved in 3 archaebacteria (see [53]): *Desulfurococcus mobilis* [54], *Thermoproteus tenax* [55] and *Thermofilum pendens* [56]. Although there could be post-transcriptional C-to-U RNA editing (see [57]) of U-G-C-C(U) to U-G-U-U, or even translocation of the peptidyl transfer site (e.g. from ψ^{746} or ψ^{1917} to ψ^{2555}) during the course of evolution of LSU rRNA, it is also possible that different or even multiple ψ sites participate in acyltransfer in different ribosomes. Because a Domain II sequence (m^{1}G-ψ^{746}-m^{5}U) in the peptidyl transferase center of *E. coli* LSU rRNA is encoded by U(C)-G-U in the aforementioned archaebacteria, a G-to-U RNA editing event would be required to produce the ψ^{746} homologue in these cases, but because a Domain IV sequence (C-mU-A-ψ^{1917}) in the peptidyl transferase center of *E. coli* LSU rRNA is encoded by C-U-C-U in the three archaebacteria mentioned above, direct conversion of U to the ψ^{1917} homologue could occur as in *E. coli*.

In summary, there is direct evidence, where information is available, that several ψ residues occur in the peptidyl-transfer site of prokaryotic and eukaryotic ri-

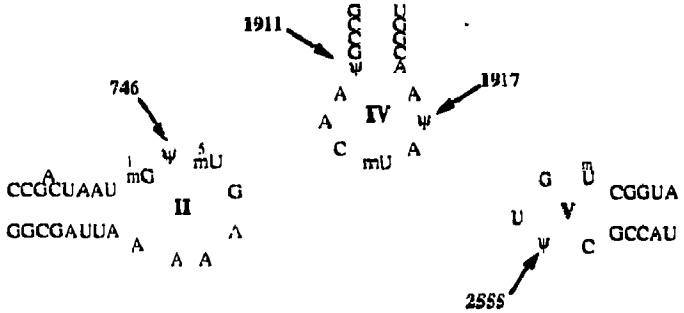

Fig. 2. A schematic drawing of the three ψ-rich hairpin loop structures known to be present in the peptidyl-transfer center of *E. coli* 23 S rRNA [40–50]. In addition to the three ψ residues in the loops from Domains II (position 716), IV (position 1917) and V (position 2555), there is a fourth residue in the stem of the hairpin from Domain IV (position 1911), and together these account for most of the 4 or 5 ψ residues present in *E. coli* 23 S rRNA [58].

bosomes. For example, there are 4 or 5 ψ residues per mole of *E. coli* LSU rRNA [58] and most if not all are in the peptidyl transfer center (Fig. 2). When taken together with the likelihood that ψ shares the remarkable acyltransfer properties of 1-acetyluracil and 1-acetylthymine (Fig. 1) [19], it is attractive to consider a role for ψ in the ribosome-mediated synthesis of peptide bonds. The ψ residues in RNA may be analagous to catalytically active histidyl residues in proteins, e.g. *N*-acetylimidazole and 1-acetyluracil have similar acetylating activities [19,59]. However, just as most histidines in proteins are not catalytically active, it is unlikely that most pseudouridines in RNA are catalytically active.

Nonetheless, it is of note in the latter context that, by way of accounting for a closely parallel (~10-fold) variation [36,58,60] in the quantities of $O^{2'}$-methylated nucleosides and ψ in the rRNAs of pro- and eukaryotes, we once suggested a possible role for the N-1 position of ψ in the transfer of methyl groups from *S*-adenosylmethionine to the $O^{2'}$-hydroxyl groups in rRNA [25,36]. Pseudouridine has been aptly dubbed the 'Cinderella' of modified nucleosides [39] and we have tried to show in this report why we feel that study of the aminoacyl-transfer potential of ψ, a property not implicity shared by any other of the known modified or classical nucleosides, is overdue [19]. In this context, we have begun comprehensive experimentation on the proposal outlined in this paper. The finding that U-to-ψ conversion in tRNA has pleiotropic effects of much greater magnitude than previously suspected [61] is fully consistent with our view about the potential importance of U-to-ψ conversion in rRNA.

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